Endosymbiotic *Wolbachia* of parasitic filarial nematodes as drug targets

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The parasitic nematodes *Wuchereria bancrofti*, *Brugia malayi* and *B. timori* cause a dreadful disease in humans known as lymphatic filariasis, which afflicts more than 120 million people worldwide. As per recent epidemiologic estimates on prevalence of *W. bancrofti* and *B. malayi*, about 428 million people are at risk, with 28 million microfilaria carriers and 21 million clinical cases spread out in 13 States and 5 Union Territories of India. The Indian subcontinent that comprises Bangladesh, India, Maldives, Nepal and Sri Lanka harbours 50 per cent of the world’s lymphatic filarial disease burden. Recently, an endobacterium of *Wolbachia* species that belongs to the family Rickettsiaceae was found in all life cycle stages of these nematodes and the transmission is exclusively vertical through the embryonic stages of the female worms. People with filariasis have been exposed to these *Wolbachia* bacteria or their proteins by the natural killing of parasites. *Wolbachia* have also been identified occasionally in body fluids of infected patients. Evidence suggests that these *Wolbachia* are mutualistic symbionts and can be cured from the nematodes by several antibiotics having antirickettsial properties. Treatment of nematodes with tetracyclines affect *Wolbachia* and they get cleared from worm tissues; and this elimination causes reproductive abnormalities in worms and affect worm’s embryogenesis, resulting in sterility. Although it is impractical, prolonged treatment with doxycycline significantly reduces the numbers of microfilaria in circulation, which is an important strategy to control transmission of filariasis by mosquito vectors. In this review, the current knowledge of *Wolbachia* as a drug target and potential ways to reduce the infection through anti-*Wolbachia* treatments is discussed.

**Key words** Antibiotics - *Brugia malayi* - diethylcarbamazine - doxycycline - embryogenesis - endobacteria - filariasis - *Onchocerca volvulus* - rickettsia - *Wolbachia* - *Wuchereria bancrofti*

**Biology of filarial Wolbachia**

*Wolbachia* of filarial nematodes (Fig.) are the obligate intracellular alpha-proteobacteria and have some resemblances with insect *Wolbachia*. They were first found in hypodermal tissues of lateral chords, uterine wall and in embryos of filarial nematodes (Figs A, B), embedded as single or multiple organisms in host-derived vacuoles. They attain different shapes (oval, round or rod-shaped) and are 0.6-1.5 µm in size. The body is covered with a double membrane enclosing the cytoplasm rich in dense ribosomes (Fig. C). In late 1970s, two groups first identified these endobacteria in filarial worms and speculated that
**Fig.** Adult *Brugia malayi* filarial nematode worms (A & B) and a section of female worm’s hypodermis under transmission electron microscopy showing (arrow) *Wolbachia* endobacteria (C). Magnifications, 10x (A, B). Scale bars, 1 cm (A, B) and 1 µm (C).
antibiotics could be used to treat filarial infections\textsuperscript{1-3}. All life cycle stages of filarial worms are infected with these bacteria, but the intensity of the infections varies between the life cycle stages\textsuperscript{4,5}, and appears that they have their own developmental life cycle within the worms which is yet to be clearly defined. Several filarial nematodes have been shown to contain these bacteria\textsuperscript{6,7} and, interestingly, only a few species (for example: \textit{Loa loa}, \textit{Acanthocheilonema viteae}, \textit{Setaria equina} and \textit{Onchocerca flexuosa}) do not carry these bacteria\textsuperscript{7,9}. It is common in several other bacterial infections, that they can crossover from one host to another. Studies suggest that \textit{Wolbachia} in filarial nematodes have coexisted for several million years and have not crossed over from their intermediate hosts (mosquitos for example) recently\textsuperscript{10,11}. However, loss of \textit{Wolbachia} across the nematode family was reported during their evolution\textsuperscript{7}. Identification of new molecules in drug discovery research against filarial nematodes was boosted by the observation that \textit{Wolbachia} can be used as a drug target and thus hold great promise towards therapeutic options available for filariasis treatment.

\textbf{Wolbachia as a target for therapy in animal models}

We and others\textsuperscript{12,13} have shown that antibiotics active against Rickettsiaceae, particularly the tetracyclines, rifampicin and chloramphenicol, were effective in reducing the filarial larval molt (from L3 to L4) and their development \textit{in vitro}. In contrast, effect of tetracycline analogues lacking antirickettsial properties also affected larval molting indicating that the drug might have other pharmacological effects on worms\textsuperscript{14}. In \textit{Brugia} infected animals, tetracycline was prophylactic and affected the molting of infective larvae\textsuperscript{15-17}, and caused distortion of male/female sex-ratios\textsuperscript{15}. Sex-ratio bias by \textit{Wolbachia} has a positive influence on insect population. Accordingly, \textit{Wolbachia} may produce sex-ratio distortion during nematode development as well. This scenario would have profound implications in filarial biology as more females survive to produce millions of microfilariae and the role of males is restricted to reproduction.

Antibiotics also affect adult filarial worms \textit{in vitro} by reducing their ability to produce microfilariae and their viability\textsuperscript{18}. Several reports have shown effects of antibiotics on filarial nematodes in experimental animal models\textsuperscript{16,17,19-23}. More importantly members of tetracycline family (tetracycline, oxytetracycline, doxycycline, and minocycline) were found to be effective against worms. These antibiotics also affect \textit{Wolbachia} after treatment\textsuperscript{18-23}. Modes of action of these antibiotics are generally on bacterial RNA polymerases, protein synthesis, and other processes, and these agents may affect similar pathways in both worms and their \textit{Wolbachia}. In several nematode worm infections these antibiotics have multiple effects on worm growth and development; worm fertility (particularly female worm embryogenesis) and worm survival, with evidence suggesting that prolonged treatment can be detrimental to worms\textsuperscript{19,21}. Moreover, when microfilaraemic animals were treated, their microfilarial numbers were considerably reduced in the circulation\textsuperscript{19}. In contrast, in animals infected with aposymbiotic \textit{A. viteae} worms, which do not carry these bacteria, similar long-term treatment had no effect on worm biology and development\textsuperscript{19}, suggesting that these bacteria play a very important role in the growth and reproduction of the filarial worms that harbour them. The combination studies with rifampicin in animal models have been found promising to achieve acceptable short-term regimen plans with doxycycline\textsuperscript{24}.

Interestingly, in addition to anti-\textit{Wolbachia} properties\textsuperscript{18-23}, tetracyclines markedly affected the normal embryogenesis profiles by causing damage and degeneration of intrauterine embryos\textsuperscript{18-23,25}. Polymerase chain reaction (PCR) assay also confirmed the clearance of \textit{Wolbachia} DNA after prolonged therapy\textsuperscript{20,23}. The reduction or clearance of bacterial-specific hsp60 and \textit{Wolbachia} surface protein (WSP) as determined by immunohistochemical staining indicated the absence or clearance of \textit{Wolbachia} in treated worms\textsuperscript{20,26}.

\textbf{Wolbachia as a target of therapy against pathogenic human filarial infections}

The availability of safe drug doxycycline has encouraged clinical investigators to test their hypothesis that elimination of \textit{Wolbachia} is beneficial in reducing the human filarial infections. The first clinical trials were done in people having onchocerciasis infections. A 6 wk course of daily doxycycline treatment (100 mg/day) depleted \textit{Wolbachia} in worms, and caused extensive
degeneration of embryos by 4 months post-treatment\textsuperscript{27}. The worms became sterile after the loss of \textit{Wolbachia}, and infected individuals also had significantly fewer or no microfilaremia\textsuperscript{27}. The combination therapy with doxycycline and ivermectin also remarkably reduced microfilaremia following reductions in \textit{Wolbachia} in worms\textsuperscript{28,29}. Similar effects were observed in \textit{W. bancrofti}-infected patients after multiple doses of doxycycline (200 mg/day for 6 wk)\textsuperscript{30}. In this study, patients were treated with doxycycline followed by a single dose of ivermectin. Doxycycline treatment alone reduced \textit{Wolbachia} numbers (96\%) after 4 months of treatment, followed by 99 per cent reductions in number of microfilariae by one year of treatment. It would be interesting to see whether \textit{Wolbachia} can repopulate in these worms after cessation of antibiotic therapy. Additional studies are needed to effectively measure macrofilaricidal activity of these drugs in such clinical studies. Interestingly, doxycycline treatment showed no effect on \textit{Loa loa} (free of \textit{Wolbachia}) infections in humans\textsuperscript{31}.

Despite this demonstrated efficacy, multi-dose antibiotic therapy and their mass treatment regimens remain impractical especially in children and pregnant women\textsuperscript{32}. Therefore, the efficacy of short-term antibiotic treatments along with antifilarial drug combinations such as diethylcarbamazine (DEC) and albendazole in various endemic countries remains to be tested.

Other than doxycycline treatment studies in selective populations carrying onchocerciasis, loaisis, or lymphatic filariasis\textsuperscript{27-31}, no clinical trials with this potent antibiotic has been reported in other endemic areas. Therefore, it is still premature to have a consensus regarding the effective universal dosage and duration of treatment for either microfilaria clearance or adult worm sterility. Moreover, the results of treatment may be affected by the immunological status of the host, age, host susceptibility and total worm burden. The filarial \textit{Wolbachia} genome sequencing has been recently completed\textsuperscript{33,34} and several new targets necessary for the bacteria are being identified. These might lead to investigate a new class of anti-\textit{Wolbachia} drugs that benefit filarial chemotherapy research (B. Slatko & J. Foster, personal communication).

**Perspective**

Human filariasis continued to be a major public health problem in parts of Indian subcontinent and other tropical areas of the world as a vector borne communicable disease\textsuperscript{35-37}. The current antifilarial therapies are restricted to DEC or ivermectin in combination with albendazole\textsuperscript{38-42}. Identification of new parasite molecules, biological targets (for example \textit{Wolbachia}) and lead compounds against them are under way to minimize or control filariasis. The results emerged from experimental animal models and limited human studies are very promising; and targeting \textit{Wolbachia} might be a strategic new approach to treat filariasis. This concept has been extensively reviewed recently\textsuperscript{43-45}. More potent prophylactic antibiotic drugs or antibacterial agents in eliminating \textit{Wolbachia} followed by parasites may be identified within pharmaceutical research platforms. For example, tigecycline (Wyeth Pharmaceuticals), an injectable class of tetracycline derivative that inhibits bacterial protein synthesis and cell growth which is under clinical trials can be tested against experimental filarial infections\textsuperscript{46}. Another promising approach, exploiting novel drug delivery such as liposomes, is based on the positive modulation of pharmacokinetics of drugs\textsuperscript{37,49}. Incorporation of potent antibiotics into liposomes can consequently increase bioavailability and prolonged drug circulation time, usually allows for lowering of the dosage, and hence diminish adverse toxicities associated with therapy\textsuperscript{47-49}. Antibiotics also have anti-inflammatory properties and therefore, it is possible to reduce post-treatment reactions (for example, ocular lesions in onchocerciasis and Mazzotti reactions) by administering these before standard antifilarial therapy. Targeting inclusion bodies of several pathogenic protozoans has always been an area of interest to develop novel therapeutics\textsuperscript{50-51}. Similarly, the evidence presented so far with filarial \textit{Wolbachia} strongly suggests that these worm-specific endobacteria emerge as one of the targets for reducing worm burden, fertility and transmission. Because of drug resistance and possible toxicity, general antibiotic use is not an option for widespread use. More research is needed to explore new biochemical pathways in \textit{Wolbachia} life cycle that
are important for parasites to survive and new enzymes involved in Wolbachia growth and development; and once identified, their inhibitors might be the silver bullets to use in filarial therapy.

References


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